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Acute Toxicity of 54 Industrial Chemicals to Sheepshead Minnows (*Cyprinodon variegatus*)

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Marine toxicity tests were conducted at EG&G Bionomics Marine Research Laboratory (BMRL) with sheepshead minnows, Cyprinodon variegatus, and 54 chemicals.

Toxicity tests were conducted to develop a data base from which water quality criteria could be established and to help determine priorities for further research efforts. The chemicals were generally those used by industry in relatively large quantities that pose potential or suspected environmental hazards, such as, chlorinated benzenes, phenols, and phthalates.

MATERIALS AND METHODS

Chemicals tested were purchased from chemical supply companies and were analytical grade with a minimum purity of 80%. Nominal concentrations are given here as mg of chemical per ℓ of seawater or ppm.

Juvenile sheepshead minnows tested were 8-15 mm standard length (14-28 days old posthatch) and were hatched and reared at BMRL or obtained from the EPA's Environmental Research Laboratory, Gulf Breeze, FL. Fish were maintained in the laboratory in flowing, filtered (10-μm) seawater of ambient salinity from 10-31 °/oo, and temperature from 25-31°C. Fish were fed 24-h Artemia salina nauplii (San Francisco Bay Brand) daily until they were used as test animals. Fish mortality during the 48-h period immediately before each test was less than 3% and all fish tested appeared to be in good condition.

Methods for the 96-h static tests were those described in "Methods for acute toxicity tests with fish, macroinvertebrates, and amphibians" (U.S. EPA 1975). Tests were conducted in either 4-ℓ glass jars that contained 3 ℓ of test solution or 19-ℓ glass jars that contained 15 ℓ. All dilution water was filtered (5-μm), natural seawater of ambient salinity. Ten fish were tested per container. There was no aeration.

Concentrations for definitive tests were based on the results of range-finding tests and were prepared

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either by direct addition of weighed amounts of test material to seawater in the test containers or by pipetting appropriate volumes of weighed amounts of the test material mixed with a solvent/carrier (reagent grade acetone, purified triethylene glycol, or deionized water). In tests where solvent/carriers were used, the control containers received a volume of the solvent/carrier equivalent to the highest volume added to any test concentration. A test was not considered acceptable if control mortality exceeded 10%.

For most tests, mortality was recorded at 24, 48, 72, and 96 h; the dissolved oxygen concentration was measured in each test container at initiation of testing and daily thereafter; pH was measured in the control and the low and high test concentrations at the initiation and after 96 h of testing.

Based on the results of the tests, 24-, 48-, 72-, and 96-h LC50's and their 95% confidence limits were calculated when possible. Calculations were performed with a Hewlett-Packard Model 9815A calculator programmed (STEPHAN 1977 and 1978 personal communication) to scan the data base and to select the most appropriate of three statistical methods in the following order: moving average angle analysis, probit analysis, or binomial probability. The method selected was determined by the characteristics of the data, that is, the presence or absence of 0% and 100% mortality and the number of concentrations in which >0%<100% mortality occurred. The computer scanned the data, identified the most suited method, and performed the analyses. No correction was made for control mortality.

RESULTS AND DISCUSSION

The acute toxicity of the 54 chemicals to sheepshead minnows varied widely (Table 1). The most toxic chemical groups were the chlorinated phenols and the chlorinated benzenes with 96-h LC50's (based on nominal concentrations) ranging from 1.7-5.4 ppm and 0.8-21 ppm, respectively. The most toxic chemical tested appeared to be 1,2,4,5-tetrachlorobenzene with a 96-h LC50 of 0.8 ppm (95% confidence limits of 0.7-1.1 ppm). The 96-h LC50 for pentachlorobenzene was the same (0.8 ppm; 95% confidence limits of 0.4-1.8 ppm), but its effect early in the exposure was not as severe as the effect of 1,2,4,5-tetrachlorobenzene. The majority of the chemicals had 96-h LC50's in the range of 10-500 ppm and were considered to be slightly toxic to practically nontoxic. Ten of the chemicals had

Table 1. Acute toxicity of 54 priority pollutant chemicals to sheepshead minnows (Cyprinodon variegatus). The 96-hour, static toxicity tests were conducted in natural seawater. Calculated LC50's, based on nominal concentrations, are presented with the 95% confidence limits given in parentheses.

Chemical	LC50 (95% confidence limits) ppm				No observed effect concentration (ppm)
	24-hour	48-hour	72-hour	96-hour	
acenaphthene	3.7 (1.7-6.2)	2.3 (1.4-3.4)	-[a]	2.2 (1.7-2.8)	1.0
antimony	>6.2<8.3 ---	>6.2<8.3 ---	>6.2<8.3 ---	>6.2<8.3 ---	6.2
chlorobenzene	>20 ---	8.9 (6.9-11)	-[a]	10 (8.8-12)	6.2
1,2-dichlorobenzene	>9.7<13 ---	9.3 (8.4-10)	9.7 (9.0-10)	9.7 (9.0-10)	9.7
1,3-dichlorobenzene	8.5 (7.5-9.6)	8.0 (7.0-9.0)	8.0 (7.0-9.0)	7.8 (6.8-8.7)	4.2
1,4-dichlorobenzene	>7.5-10 ---	7.2 (6.5-7.7)	7.4 (6.6-7.9)	7.4 (6.8-7.9)	5.6
1,2,4-trichloro-benzene	>47 ---	>47 ---	>47 ---	21 (17-26)	15
1,2,3,5-tetra-chlorobenzene	>7.5 ---	5.6 (5.2-6.0)	4.7 (3.9-5.5)	3.7 (3.3-4.1)	1.0
1,2,4,5-tetra-chlorobenzene	>1.8 ---	0.9 (0.7-1.2)	0.8 (0.7-1.1)	0.8 (0.7-1.1)	0.3

[a]No observation made.

Table 1 (continued).

Chemical	LC50 (95% confidence limits) ppm 24-hour	48-hour	72-hour	96-hour	No observed effect concentration (ppm)
pentachlorobenzene	>32 ---	9.6 (7.0-12)	>3.2<10 ---	0.8 (0.4-1.8)	0.3
1,2-dichloroethane	>130<230 ---	>130<230 ---	>130<230 ---	>130<230 ---	130
1,1,2,2-tetra-chloroethane	19 (14-120)	16 (12-20)	13 (5.1-33)	12 (4.7-32)	<8.8
1,1,1-trichloro-ethane	68 (57-79)	71 (60-81)	71 (60-81)	71 (60-81)	43
1,1,1,2,2-penta-chlorethane	120 (110-130)	120 (110-150)	120 (110-150)	116 (100-140)	30
1,1,1,2,2,2-hexa-chloroethane	3.1 (2.4-4.1)	2.8 (2.2-3.6)	2.4 (1.9-3.1)	2.4 (1.9-3.1)	1.0
1-chloronapthalene	3.4 (3.2-4.4)	2.5 (1.9-3.1)	2.4 (1.8-3.0)	2.4 (1.8-3.0)	1.2
octachloro-naphthalene	>560	>560	>560	>560	560
4-chlorophenol	5.7 (4.9-6.4)	5.4 (4.3-7.1)	5.4 (4.3-7.1)	5.4 (4.3-7.1)	3.2
2,4,5-trichloro-phenol	2.4 (1.9-3.0)	1.7 (0.9-3.2)	1.7 (0.9-3.1)	1.7 (0.9-3.1)	1.0

TABLE 1 (continued).

Chemical	LC50 (95% confidence limits) ppm 24-hour	48-hour	72-hour	96-hour	No observed effect concentration (ppm)
2,3,5,6-tetra-chlorophenol	2.0 (1.5-2.5)	2.0 (1.5-2.5)	2.0 (1.5-2.5)	1.9 (1.4-2.4)	1.0
1,1-dichloroethylene	250 (200-340)	250 (200-340)	250 (200-340)	250 (200-340)	80
1,3-dichloropropane	87 (77-100)	87 (77-100)	87 (77-100)	87 (77-100)	38
1,3-dichloropropene	>6.8<9.2 ---	3.3 (2.6-4.3)	2.2 (1.5-2.8)	1.8 (0.7-4.5)	1.2
2,3-dinitrotoluene	>7.5 ---	5.0 (4.4-5.5)	2.9 (1.8-6.4)	2.3 (1.4-3.4)	0.6
ethylbenzene	300 (250-340)	360 (310-440)	320 (270-380)	280 (260-290)	88
fluoranthene	>560 ---	>560 ---	>560 ---	>560 ---	560
bromoform	19 (16-23)	19 (16-23)	18 (15-21)	18 (15-21)	2.9
methylene chloride	370 (330-410)	360 (310-410)	360 (310-410)	330 (280-380)	130
isophorone	>170<300 ---	>170<300 ---	>170<300 ---	>170<300 ---	170

Table 1 (continued).

Chemical	LC50 (95% confidence limits) ppm 24-hour	48-hour	72-hour	96-hour	No observed effect concentration (ppm)
nitrobenzene	>120 ---	>120 ---	>120 ---	59 (47-69)	22
4-nitrophenol	28 (25-31)	28 (25-31)	27 (23-31)	27 (23-31)	24
2,4-dinitrophenol	42 (34-54)	32 (24-40)	32 (24-40)	29 (26-34)	10
2,4,4-trinitrophenol	130 (100-170)	130 (100-170)	130 (100-170)	130 (100-170)	100
butyl benzyl phthalate	380 (310-430)	420 (380-460)	430 (390-470)	440 (410-470)	360
diethyl phthalate	>69 ---	38 (33-44)	36 (29-47)	30 (23-38)	22
dimethyl phthalate	>120 ---	58 (47-68)	58 (47-68)	58 (47-68)	21
selenium	56 (15-200)	26 (16-40)	13 (9.8-16)	6.7 (5.1-8.6)	2.0
silver[a]	>54<110	>64<110	-[b]	58 (48-67)	6.4
tetrachloro-ethylene	>52 ---	>52 ---	-[b]	>29<52 ---	29

[a]A similar test conducted later had values an order of magnitude lower. The discrepancy is probably related to silver complexing with Cl- in seawater.
[b]No observation made.

Table 1 (continued).

Chemical	LC50 (95% confidence limits) ppm 24-hour	48-hour	72-hour	96-hour	No observed effect concentration (ppm)
thallium	>45 ---	>26<45 ---	-[b]	21 (17-27)	14
toluene	>280<480 ---	>280<480 ---	>280<480 ---	>280<480 ---	280
bis-(2-ethylhexyl) phthalate	>550 ---	>550 ---	>550 ---	>550 ---	550
barium	>500 ---	>500 ---	>500 ---	>500 ---	500
camphene	1.8 (1.0-3.2)	2.0 (1.6-2.4)	2.0 (1.6-2.4)	1.9 (1.6-2.2)	1.0
p-cymene	56 (32-100)	50 (38-68)	48 (36-64)	48 (36-64)	10
n-decane	>500 ---	>500 ---	>500 ---	>500 ---	500
n-docosane	>500 ---	>500 ---	>500 ---	>500 ---	500
dibenzofuran	>3.2 ---	>3.2 ---	3.1 (2.6-4.2)	1.8 (1.0-3.2)	1.0
diethanolamine	>540 ---	>540 ---	>540 ---	>540 ---	>540

Table 1 (continued).

Chemical	LC50 (95% confidence limits) ppm 24-hour	48-hour	72-hour	96-hour	No observed effect concentration (ppm)
diphenyl ether	2.4 (1.8-3.2)	2.4 (1.8-3.2)	2.4 (1.8-3.2)	2.4 (1.8-3.2)	1.0
n-dibutyl ether	>430 ---	>430 ---	>430 ---	>430 ---	430
methyl ethyl ketone	>400 ---	>400 ---	>400 ---	>400 ---	400
sodium fluoride	>500 ---	>500 ---	>500 ---	>500 ---	500
styrene	9.1 (5.1-16)	9.1 (5.1-16)	9.1 (5.1-16)	9.1 (5.1-16)	5.1

no apparent effect on sheepshead minnows at the highest concentrations tested.

Many of the chemicals were insoluble in seawater and either floated upon the water surface or formed globules on the bottoms of the test containers. In these cases, calculated LC50's based on the nominal concentrations should be interpreted as comparative rankings of toxicities to sheepshead minnows under uniform conditions rather than a fixed value.

Results of these tests with sheepshead minnows compared to those of related tests conducted with the water flea, Daphnia magna, (LEBLANC 1980) showed a general correlation in the order of toxicity of the chemical groups tested, the most toxic groups to both animals being the chlorinated benzenes and chlorinated phenols. Also, interestingly, sheepshead minnows were as sensitive or more than were the daphnids to 28 of the 54 chemicals.

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